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EXTRACTION OF POLYHYDROXY COMPOUNDS FROM DILUTE AQUEOUS SOLUTIONS BY CYCLIC ACETAL FORMATION

III. THE CONTINUOUS EXTRACTION OF GLYCEROL[1]

BY R. R. TINK[2] AND J. M. ROXBURGH

Abstract

Data are given for the extraction of glycerol as the cyclic *n*-butyral in a 14 stage countercurrent liquid–liquid extractor of the Scheibel type. The effect of variations in feed concentration, ratio of *n*-butyraldehyde to water, total throughput, temperature, acid concentration, and stirring rate on the efficiency of the reaction is shown. The change in glycerol concentration as the aqueous phase passes through the column was determined. Experiments on a similar column with two stages show that a single long column is preferable to several short ones (totalling the same number of stages) operated with fresh solvent in each. A five to six stage column is shown to be equivalent to a single batch extraction under similar conditions.

Introduction

Data for the extraction of glycerol as the cyclic acetal with excess *n*-butyraldehyde as the solvent in a batch process have been given in a previous paper in this series (4). In this paper, similar data for the countercurrent liquid–liquid extraction process with Scheibel columns are presented.

Although liquid–liquid extraction in the ordinary sense has been treated mathematically, and useful relations developed, no such treatment appears in the literature for the type of extraction-reaction to be considered here, where the rate of extraction is largely dependent on the rate of a relatively slow chemical reaction. No wholly satisfactory relation follows directly from a simple kinetic treatment (5) and the effect of most of the variables on the amount of extraction cannot be expressed in the form of equations which can be confirmed by experiment. In this paper, therefore, the data are presented empirically.

Experimental

A laboratory model 1 in. Scheibel column with 14 stages was used for most of these experiments (1). The only alterations made were the addition of a water jacket for temperature control and of sampling stopcocks at four points

[1] *Manuscript received December 18, 1950.*
Contribution from the National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Sask. Issued as Paper No. 114 on the Industrial Utilization of Wastes and Surpluses and as N.R.C. No. 2417.
[2] *Present address, McGill University, Montreal, Que.*

down the column. Constant flow of the aqueous phase was obtained by maintaining a constant head over a calibrated capillary. An adjustable stroke, stainless steel, bellows pump was used to regulate the flow of *n*–butyraldehyde. The interface was maintained by an electrical contact which operated a centrifugal pump (through a relay) taking suction from the bottom of the column. A schematic diagram of the column and its controls is shown in Fig 1. The two stage column used for some of the experiments was similar, but there were no sampling stopcocks fitted.

All experiments were continued until no significant change in the composition of the raffinate or of the extract could be detected in samples taken one hour apart. In general, experiments were run for at least six hours in the

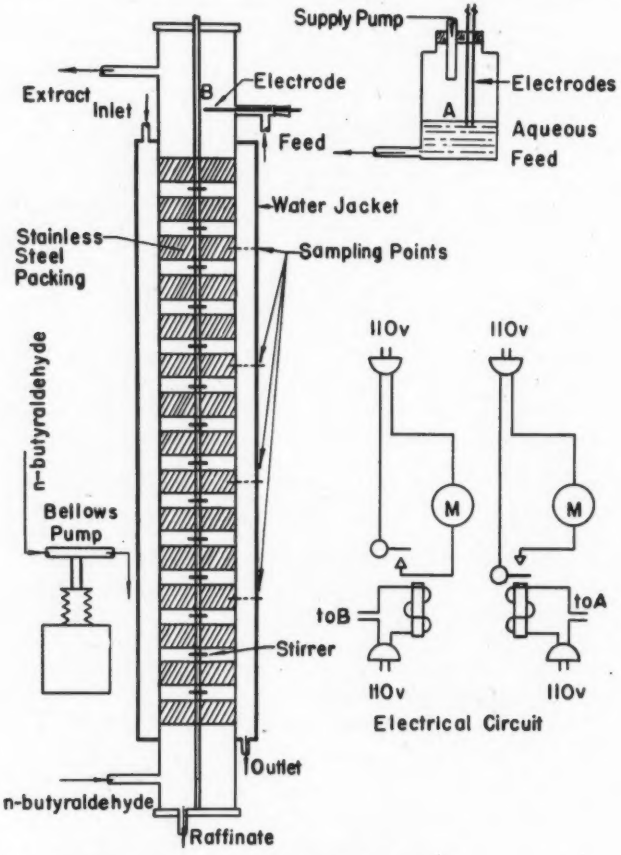

FIG. 1. *Fourteen stage column with flow and level control.*
A – electrodes controlling aqueous feed head.
B – electrodes controlling column interface level.
M – motors of stainless steel centrifugal pumps for aqueous feed and raffinate.

long column and four hours in the short one. All glycerol concentrations are given as total glycerol, free and combined as acetal, and were determined as described in previous papers (3, 4).

A standard set of conditions for the variables was adopted, and one variable changed, over a range, in each set of experiments. Standard conditions were:

Continuous phase	water
Temperature	30°C.
Total flow rate	1.50 liters per hour
Ratio *n*-butyraldehyde to water	0.50
Catalyst	0.378 normal HCl
Feed composition	4% glycerol
Stirring	1620 r.p.m.

Results and Discussion

The results obtained with the 14 stage column are summarized in Table I and some are presented graphically for clarity in Figs. 2 to 5. The effects of the variables can be compared, qualitatively at least, with similar data presented previously for the batch process (4).

The initial concentration of glycerol in the feed does not affect the degree of extraction obtained (Fig. 2); this applies to the batch process also, so that in either case, increasing the glycerol content of the feed (by evaporation for example) serves to reduce the amount of both phases to be transferred, to reduce

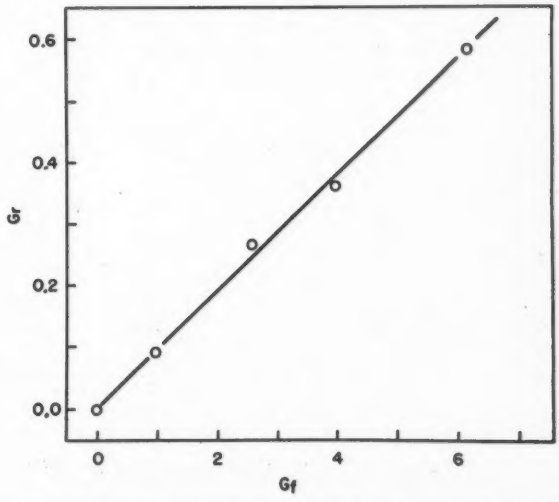

FIG. 2. *Effect of glycerol concentration in the feed (G_f) on the concentration of glycerol in the raffinate, free and combined (G_r). Concentrations in gm. per 100 ml.*

TABLE I

EFFECT OF THE CONCENTRATION OF THE FEED, TOTAL THROUGHPUT, RATIO OF THE FEEDS, ACID CONCENTRATION, TEMPERATURE, AND STIRRING RATE ON THE EXTRACTION OF GLYCEROL WITH *n*-BUTYRALDEHYDE IN A 14 STAGE SCHEIBEL COLUMN. CONTINUOUS PHASE = WATER

Expt. No.	Concentration of glycerol in feed (gm./100 ml.)	Total feed rate (l./hr.)	Ratio of *n*-butyral-dehyde to water	Acid conc. (*N*)	Temperature (°C.)	Stirring rate (r.p.m.)	Raffinate		Extract	
							*	**	*	**
50	0.97	1.50	0.50	0.378	30	1620	0.091	1.05	2.10	0.43
102	2.57						0.268	1.08	5.31	0.44
42	4.00						0.360	1.12	7.90	0.44
54	6.19						0.580	1.10	11.8	0.44
62	4.00	0.69	0.50	0.378	30	1620	0.243	0.48	8.39	0.20
42		1.50					0.360	1.12	7.90	0.44
74		2.07					0.52	1.40	7.74	0.65
80		2.43					0.877	1.68	7.12	0.74
58		3.00					1.20	2.04	6.01	0.89
66	4.00	1.23	0.23	0.378	30	1620	0.804	1.10	14.7	0.81
86		1.40	0.40				0.640	1.06	9.64	0.33
42		1.50	0.50				0.360	1.12	7.90	0.44
70		2.05	1.05				0.304	1.05	4.21	0.94
—		1.10	0.10				3.55	1.09	—	0.00
94	4.00	1.50	0.50	0.1032	30	1620	1.88	1.10	3.84	0.44
98				0.1900			1.01	1.11	6.10	0.44
90				0.2735			0.575	1.11	7.35	0.44
42				0.378			0.360	1.12	7.90	0.44
106	4.00	1.50	0.50	0.378	20.5	1620	0.91	1.07	6.85	0.42
42					30.0		0.360	1.12	7.90	0.44
110					41.6		0.264	1.02	8.60	0.46
112	4.00	1.50	0.50	0.378	30	800	0.38	1.02	8.39	0.44
114						1200	0.35	1.10	8.21	0.44
42						1620	0.36	1.12	7.90	0.44
116						1910	0.36	1.10	7.89	0.44

* *Glycerol conc., gm./100 ml.;* ** *Rate, l./hr.*

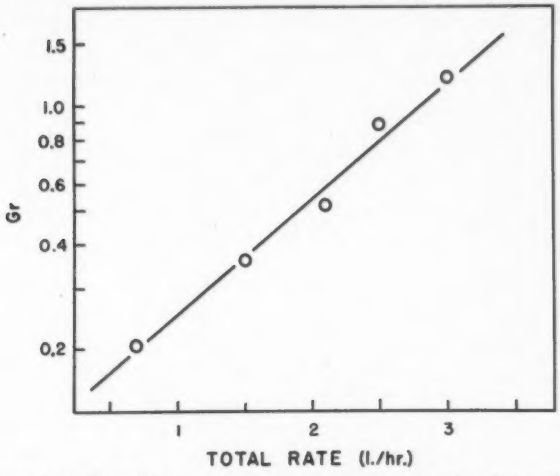

FIG. 3. *Effect of total throughput on the extraction of glycerol with n-butyraldehyde. Feed 4% glycerol, 0.378 N hydrochloric acid.*

losses of *n*-butyraldehyde in proportion to the glycerol recovered, and to increase the extract concentration proportionally.

The efficiency of the extraction in the column is independent of the rate of stirring in the range investigated. By analogy with results reported for a similar column used for solvent extraction (2) a decrease in efficiency is to be expected at much lower and at much higher stirring rates.

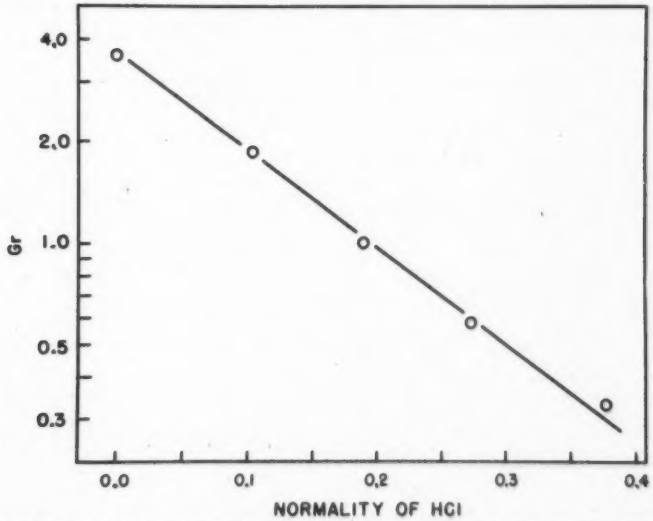

FIG. 4. *Effect of catalyst concentration on the extraction of glycerol with n-butyraldehyde. Feed, 4% glycerol.*

* Glycerol conc., gm./100 ml.; ** Rate, l./hr.

Total throughput, which determines the time of contact in the column, shows a logarithmic relation to the concentration of glycerol in the raffinate (Fig. 3) as does time in the batch process (4). In the column, the ratio of *n*–butyraldehyde to water is not independently variable, since either total throughput or one of the feed rates must be varied with it. At a constant aqueous feed rate, the efficiency of the extraction is increased by increasing the ratio (Table I). At constant throughput this effect would be even more noticeable. The improvement in efficiency is closely comparable to that observed in the batch process for the same variable.

Increased temperature increases the efficiency of the column (Table I) because of the increased rate of reaction (4). The counteracting effect of a less favorable equilibrium (4) makes the temperature effect drop off rapidly above 30° to 35°C. The optimum temperature, both for the column and for batch extraction, is governed by the time of contact between phases. If contact time is short (high total throughput in the column) then a higher temperature is desirable to obtain the advantage of the increased rate of reaction. If contact time is long, then a lower temperature should be used to benefit from the more favorable equilibrium.

The concentration of catalyst, which was shown to have a linear effect on the rate constant in the batch process (4), here shows an analogous logarithmic relation to the concentration of glycerol in the raffinate (Fig. 4). Again, as with temperature, the optimum value depends on contact time, taking into consideration the increased losses of *n*–butyraldehyde by aldol formation and oxidation at higher acid concentrations.

In Fig. 5 is shown a typical plot of glycerol concentration as a function of the number of the stage in the column. An empirical relation between log

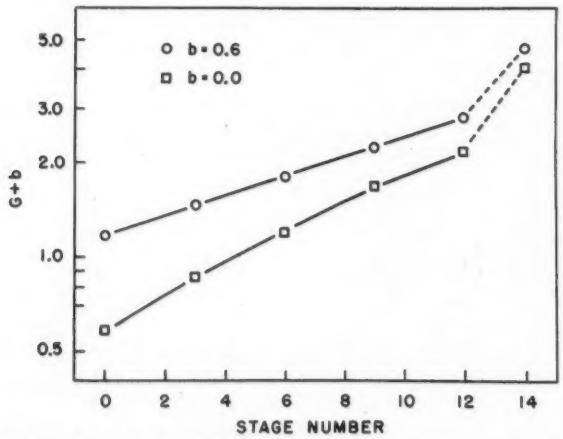

FIG. 5. *Total glycerol concentration in the aqueous phase (G) at various points in the column at equilibrium. Glycerol in the feed, 4%.*

$(G + b)$ and stage number is shown, where "G" is the aqueous glycerol concentration and "b" is a small empirical constant. The higher rate of extraction in the first two stages from the feed end of the column was often, but not always, observed. No explanation has been found.

In Table II the results of two experiments with the two stage column are shown. Conditions in the first experiment were "standard", and in the second were standard except that the concentration of glycerol in the feed was made approximately that of the raffinate from the first. Two such two stage

TABLE II

TWO STAGE COLUMN

Glycerol concentration (gm./100 ml.)			% Glycerol extracted	Ratio, *n*-butyraldehyde/ water	Material balance, %
Feed	Raffinate	Extract			
4.02	2.68	4.16	33	0.48	103
2.55	1.70	2.37	36	0.43	96

columns, operated in series with fresh *n*-butyraldehyde in each, will give 58% extraction under these conditions. The first four stages (from the feed end) of the 14 stage column extract 55 to 60% of the glycerol (Fig. 5 and other experiments), despite the fact that the carbonyl phase entering this part of the column already has a certain amount of acetal in it. Why the two stage column with fresh solvent is not more efficient is not clear. Practically, this result suggests that a single long column to obtain the desired degree of extraction is preferable to several shorter ones totalling the same number of stages and with fresh *n*-butyraldehyde in each.

A comparison of the data in this paper with results previously reported for the batch extraction (4) shows that the 14 stage column under "standard" conditions extracts about 10% more of the glycerol than a single batch extraction allowed to reach equilibrium. A batch extraction after 15 min. contact has extracted about 30% less than this column. A rough calculation from Fig. 5 shows that a five to six stage column is the equivalent of a 15 min. batch extraction under the same conditions of temperature, ratio, and acid concentration.

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CANADIAN ERUCIC ACID OILS

VII. PREPARATION OF SULPHATED AND HYDROXY ACIDS FROM RAPESEED OIL ACIDS[1]

By N. H. Grace[2] and A. Zuckerman[2]

Abstract

Crystallization of rapeseed oil acids from 10 vol. acetone at −40°C. yielded a precipitated fraction, designated as practical erucic acid, and an approximately equal fraction of soluble acids. Acid equivalent weights and iodine values were, respectively, 322–328 and 72–75 for the solid acids, and 296–308 and 120–139 for the soluble acids. Higher grade erucic acid was prepared by a preliminary crystallization at −6.7°C. to remove saturated acids or by recrystallization; pure erucic acid was obtained on fractional distillation of the esters. Practical erucic acid, on distillation, yielded as ethyl esters 74% C_{22} mono-unsaturated; 4.3% C_{18} chiefly saturated; 9.7% C_{18} and C_{20} esters, chiefly mono-unsaturated; and 12% residue of polymeric material and esters from acids with more than 22 carbon atoms.

Sulphation of the two fractions of rapeseed oil acids yielded a product comparable to sulphated oleic acid, and a more mobile oil. One part by weight of 96% sulphuric acid to two parts fatty acid was the preferred treatment; the amount of acid had a greater effect than differences in temperature or time of reaction on the properties of the sulphated oil and reconstituted acids. Hydrolysis of these products yielded a mixture of hydroxy acids separated into fractions by differential solubility in hexane. Hydroxybehenic acid was obtained on low pressure distillation of the ethyl esters. The polycondensation of hydroxybehenic acid yielded oils and balsams with molecular weights from 1400–3000; reaction with tetradecanol-1 and octadecanol-1 yielded waxes melting, respectively at 58° and 65°C.

Introduction

Canadian production of rapeseed was encouraged during the war years to meet, among other requirements, the need for marine lubricants and sulphated oils. Erucic acid is found in relatively high percentage in fatty acids prepared from the seed fats of the *Cruciferae* (13). There is an extensive literature on the preparation of erucic acid from rapeseed oil (2, 5, 9, 15). The various methods usually involve separation of the fatty acids into two fractions, one largely of erucic acid contaminated with saturated acids and small amounts of eicosenoic acid; the other (approximately equal in quantity) composed largely of eicosenoic, oleic, linoleic, and linolenic acids. One object of this investigation was the rapid and simple separation of a practical grade of erucic acid that might be used to prepare various derivatives.

While sulphated rapeseed oil has long been known and used, no reference was found to the preparation of sulphated fractions. Sulphated oleic acid is used industrially as a wetting and emulsifying agent, principally in the leather and textile industries. Availability of erucic acid from rapeseed oil acids

[1] *Manuscript received January 19, 1951.*
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. N.R.C. Number 2424.
[2] *Biochemist.*

suggested the preparation of sulphated erucic acid, which might have properties comparable to those of sulphated oleic acid. Further, the more unsaturated acid fraction of rapeseed oil acids is also readily amenable to sulphation and could be expected to provide new sulphated oils. This communication describes the sulphation of two acid fractions from rapeseed oil, the preparation of several hydroxy fatty acid fractions from sulphated acids, and the use of mixed 13 and 14 hydroxybehenic acid in the preparation of certain derivatives.

Preparation of Acid Fractions from Rapeseed Oil Acids

Fatty acids were prepared by the method of Hilditch (7), from crude and partially refined (6) rapeseed oil expelled by Prairie Vegetable Oils at Moose Jaw, Sask. Preliminary investigations on the choice of solvent, of solvent to solute ratio, and of temperature and time of cooling were done to establish optimum conditions. The preferred method involved solution of one part rapeseed oil acids in 10 volumes dry acetone and cooling for 24 hr. in a cold room at −40°C. The precipitate was filtered in the cold and the solvent removed on the water bath first under an inert atmosphere and finally under reduced pressure. The weights of precipitated and soluble acids were determined, and the iodine value and acid equivalent weights were obtained by recognized procedures.

Experimental and Results

Effects of Preliminary Precipitation and Solvent–Solute Ratio

Preliminary investigation indicated that low temperature crystallization of the triglycerides from solvent was not sufficiently selective for trierucin. Further, studies with various solvents demonstrated that reasonable separation of erucic acid from the more unsaturated components and from eicosenoic and oleic acids could be achieved by cooling solutions in petrolic ether (boiling range, 30°–60°C.), or in acetone, to −40°C. When 1-gm. samples of acids were dissolved in 20 ml. acetone in centrifuge tubes, and crystallized at −40°C. precipitation was practically complete after two hours.

Table IA shows the effects of preliminary precipitation, from acetone at a higher temperature, on the yield and iodine value of the precipitate subsequently obtained at −40°C. from the filtrate. It is apparent that preliminary cooling to −6.7°C. removed only 3.5% of acids and this precipitate had a lower iodine value than the following crop of crystals, indicating appreciable removal of more saturated acids. Preliminary cooling to temperatures lower than −6.7°C. reduced final yield without any increase in quality (the theoretical iodine value for erucic acid is 74.98).

Solutions with the various solvent–solute ratios described in Table IB were cooled to −6.7°C. and the resulting precipitates removed. Subsequent cooling of the filtrates to −40°C. yielded the recorded percentages of solid acids.

TABLE I

PRECIPITATION OF RAPESEED OIL ACIDS FROM ACETONE AT −40°C.

A. EFFECTS OF PRELIMINARY PRECIPITATION AT DIFFERENT TEMPERATURES
(40 gm. of acids in 800 ml.)

Preliminary precipitation			Subsequent precipitation of filtrate at −40°C.	
Temperature, °C.	Yield, %	Iodine value	Yield, %	Iodine value
− 6.7	3.5	63.5	40.3	72.7
−12.2	21.8	72.5	25.3	71.9
−17.8	26.5	70.5	19.9	70.3
−23.3	33.3	70.3	12.6	70.4

B. EFFECTS OF SOLVENT RATIO ON YIELD AND IODINE VALUE AFTER A PRELIMINARY PRECIPITATION AT −6.7°C.
(40 gm. of acids used)

Volume of acetone for unit weight of acids	Acids precipitated from filtrate at −40°C.	
	Yield, %	Iodine value
20	42.6	71.5
17	36.8	70.0
16.5	36.5	71.8
16	38.8	73.0
15	37.8	74.1
10	35.0	74.1

Effects of Refining Treatments on Precipitation from Acetone and Ethanol

Table II describes the separation of fractions from acids of crude rapeseed oil, bleached acids from crude oil (with acetone), and fractions from acids of bleached oil either with or without removal of unsaponifiables (with acetone or alcohol). There was comparatively little difference in the quality of any of the fractions separated by acetone from the various acids, although bleaching of the oils or acids appeared to reduce the yield of the crystallized fractions. The solid acid fractions had acid equivalent weights and iodine values ranging, respectively, from 322 to 328 and 72 to 75. Removal of the unsaponifiables from acids from bleached oil yielded a product with a slightly higher iodine value.

Crystallization from absolute ethanol gave low yields and was accompanied by some esterification as shown by acid and saponification equivalent weights.

Effects of Bleaching and Recrystallization

In Table III are described separations of acid fractions from 1-kgm. samples of acids from crude and bleached oils. Samples of 100 gm. of the resulting precipitates were recrystallized from acetone at the same solvent ratio. The results indicated little difference in the behavior of acids from crude

TABLE II

EFFECTS OF PREPARATION OF RAPESEED OIL ACIDS ON CRYSTALLIZATION AT −40°C.
(Twenty gm. acids in 200 ml. solvent)

Description	Solvent	Yield,‡ %	Acid equivalent weight	Iodine value, cgm./gm.
Acids from crude rapeseed oil			309.5	106.9
Crystallized acids	Acetone	46	328.1	71.8
Soluble acids		54	295.8	132.0
Bleached acids‡‡			310.1	105.7
Crystallized acids	Acetone	40	321.9	73.9
Soluble acids		60	296.7	129.5
Acids from bleached oil‡‡			309.1	105.7
Crystallized acids	Acetone	33	326.1	71.8
Soluble acids		67	307.8	120.4
Crystallized acids	Ethanol	21	347.8*	76.8
Soluble acids		79	327.1**	111.6
Acids from bleached oil, unsaponifiables removed			309.0	106.4
Crystallized acids	Acetone	34	327.0	74.7
Soluble acids		66	299.5	121.8
Crystallized acids	Ethanol	20	345.6†	76.4
Soluble acids		80	315.4††	106.3

‡*Soluble acids determined by difference.*
‡‡*Acids and oil bleached with 4% Superfiltrol at 100°C. for 20 min.*
*Note: On saponification, * was 328.5, ** was 309.8, † was 327.5, and †† was 298.8.*

TABLE III

EFFECTS OF BLEACHING AND RECRYSTALLIZATION ON QUALITY OF ACIDS CRYSTALLIZED FROM AN ACETONE SOLUTION OF RAPESEED OIL ACIDS AT −40°C.
(Kilogram samples of acids in 10 liters acetone)

Description of acids	Yield, %	Acid equivalent weight	Iodine value, cgm./gm.	Melting point, °C.
Prepared from bleached oil				
Crystallized acids	52.7	328.3	75.4	30.5
Soluble acids	47.3*	301.8	138.6	
Recrystallization of 100 gm. of above precipitate from 10 volumes acetone				
Crystallized acids	81.5	332.0	68.2	32.0
Soluble acids	17.0	309.7	103.7	
Prepared from crude oil				
Crystallized acids	52.7	327.1	76.6	30.0
Soluble acids	47.3*	302.2	136.5	
Recrystallization of 100 gm. of above precipitate from 10 volumes acetone				
Crystallized acids	78.0	332.0	69.3	31.3
Soluble acids	20.5	311.4	106.2	

* *Soluble acids determined by difference.*

and bleached oil. Recrystallization slightly increased the melting point but effected an appreciable reduction in the iodine value. This indicated selective removal of the more unsaturated components with concentration of the saturated acids.

The data of Tables II and III indicate that separation of practical erucic acid yielded an approximately equal amount of more unsaturated acids. This fraction of rapeseed oil acids had acid equivalents and iodine values ranging, respectively, from 296 to 308 and 120 to 139, and is largely composed of oleic, linoleic, and linolenic acids with some eicosenoic and erucic acids (1, 10). The properties of the unsaturated acid fraction suggests its use in preparing synthetic drying oils and alkyd resin formulations.

Separation of Pure Erucic Acid by Fractional Distillation

Crystallization of erucic acid from 10 volumes of acetone at −40°C. yielded a crude product substantially the equivalent of that obtained by the method of Noller and Talbot (15). Laborious repeated recrystallizations yielded erucic acid of fair purity but in small yields. The preferred method for the preparation of pure erucic acid involved fractional distillation of the ethyl esters through a 47 cm. × 25 mm. Stedman column.

The results of fractional distillation of the ethyl esters of singly precipitated acids described in Table III are given in Table IV. The first three fractions

TABLE IV

FRACTIONAL DISTILLATION OF ETHYL ESTERS OF "PRACTICAL" ERUCIC ACID PREPARED BY ONE PRECIPITATION FROM ACETONE AT −40°C.

(200 gm. ethyl esters, a 25 mm. × 47 cm. Stedman column, initial pressure 23 mm. reduced to 17 mm. for collection of samples 9 to 15)

Fraction	N_D^{30}	Weight of fraction, gm.	Saponification equivalent	Iodine value, cgm./gm.
Initial ester	1.4502	200	367.1	63.3
Fraction 1	1.4370	8.6	282.2	5.1
Fraction 2	1.4468	10.9	318.9	50.6
Fraction 3	1.4481	8.5	343.1	57.8
Fractions 4 to 15	1.4500 to 1.4505	146.7	364.8	68.0
Residue		25.0		
Distillation of part of residue through 12 in. × 6 mm. Bower and Cooke column				
Distillate	1.4543		409.3	63.4
Residue			567.6	64.0

were largely composed of C_{16}, C_{18}, and C_{20} esters. Fractions 4 to 15 of closely similar refractive indices were combined, yielding a saponification equivalent and an iodine value of 364.8 and 68 (theoretical values of ethyl erucate are, respectively, 366 and 69.3). The ethyl erucate fraction amounted to 73.4% of the charge of esters and was contaminated by 3.25% of free acid calculated as erucic.

Fifty grams of the combined ethyl erucate fractions were dissolved in ethyl ether and washed several times with a total of 300 ml. of 0.1 *N* sodium carbonate solution. The carbonate washings yielded 1.1 gm. (2.2%) of a solid acid that melted at approximately 37°C. The acid material had no

peroxide value, was saturated, and showed acid and saponification equivalents, respectively, of 237 and 187. The contaminating acid was not erucic and may be attributed to slight breakdown of the ester on distillation (oxidative scission of ethyl erucate could be expected to yield a mixture of pelargonic acid and the monoethyl ester of brassylic acid; such a mixture would have theoretical acid and saponification equivalents of 215 and 143 respectively).

Table IV shows that there was a residue of 12.5% on distillation. This was darkly colored in contrast to the colorless appearance of all distilled fractions. Further distillation of part of the residue in a small column removed about 30% of slightly colored material containing 7.23% free acid calculated as erucic. The residue contained 4.7% free acid on the same basis, and was appreciably polymeric.

Sulphated Acid Fractions from Rapeseed Oil

The "practical" erucic and unsaturated acid fractions separated by one crystallization from acetone, as described, were used in the following sulphations.

The operations involved in the sulphation of fatty materials are well known (3, 4, 8, 11, 12, 17). The procedure about to be described may be considered as a simple laboratory application of well established processes. A limited range of sulphation conditions was employed as indicated in Tables V and VI. Since the erucic acid used had a melting point of 31°–33°C., sulphation temperatures were necessarily somewhat higher than would have been used with oleic acid.

The sample of fatty acid (100 gm.) was placed in a three-necked, 500 ml. flask with a stopcock sealed to its base. The flask was provided with a variable speed stirrer, a thermometer and dropping tube and was placed in a thermostatically controlled water bath, or ice bath for certain sulphations. Temporary inactivation of the thermostat and rapid cooling of the bath during the early stages of sulphation held down initial rise in temperature to a maximum of 3°.

The specified amount of 96% sulphuric acid (Tables V and VI) was run in through a dropping funnel calibrated to make delivery at a predetermined rate; the charge was allowed to react for a subsequent period of one or two hours with vigorous stirring. At the end of the reaction period, 360 ml. of sodium sulphate solution (1.05 sp. gr.) was run rapidly into the reaction mixture, which was stirred slowly for five minutes and held at about 45°C. for one hour. The lower aqueous layer was withdrawn and the mixture adjusted by means of 25.8% sodium hydroxide solution to a pH between 4.5–5, using hydrion paper as an outside indicator. The mixture was then held overnight at about 45°C. Further aqueous material was run off and the pH adjusted to 5.5–6. In a few instances, the desired pH was either not reached (Table V, No. 12) or exceeded (No. 9).

The weight of finished oil was determined, analyses made for moisture content, total alkalinity, and organically combined SO_3 (titration method) by official methods of the Sulfonated Oil Manufacturers Association (18). The melting point range, iodine values, and acid equivalent weights of the reconstituted fats were also determined.

The change in pH on dilution of the aqueous solution of some of the sulphated oils was determined by methods that had been applied to sulphated oleic acid (4).

Results

The data of Table V indicate the general results of erucic acid sulphation and the differences attributed to variations in experimental procedure. The factor of greatest consequence was undoubtedly the amount of sulphuric acid. When 25 gm. of acid was used, the resulting sulphated material tended to be thick in consistency with little flow at room temperature, though with satisfactory water solubility. When the acid content was raised to 50 gm., the sulphated product was free-flowing, though considerably more viscous than commercial sulphated oleic acid. These differences were reflected in the properties of the reconstituted fatty acids, which had consistently lower melting points and iodine values, but tended to show somewhat greater acid equivalent weights when the greater amount of sulphuric acid was used. The total alkalinity tended to be greater, and the organically combined SO_3 smaller, for the 28° treatment of short duration.

Low alkalinity was shown by No. 12, for which the sulphation involved 75 gm. sulphuric acid. This product was comparatively stiff at room temperature, differing in appearance and consistency from the sulphated products obtained with 50 gm. acid. While low alkalinity may be partially attributed to inadequate neutralization, it would appear probable that the relatively large sulphuric acid concentration promoted the extensive formation of estolides. A sulphation was attempted using 100 gm. of sulphuric acid with an equal weight of erucic acid. This yielded an even more unsatisfactory product which was not analyzed. Water separated from both these products on standing, the only instances in which this occurred in the series.

The data of Table VI give the results of sulphation of the more unsaturated fatty acid fraction from rapeseed oil acids. Here, as in Table V, the main feature of the results was the effect of 50 gm. sulphuric acid which, in comparison with the 25 gm. treatments, markedly increased organically combined SO_3 and lowered the iodine value of the reconstituted fatty acids. Excepting No. 15, there was a tendency for lower acid equivalent weight of the reconstituted fatty acids with the high sulphuric acid level, the lower sulphation temperature evidently favoring occurrence of higher acid equivalent weight. The reconstituted fatty acids were liquid at room temperature. The sulphated oils were clear and quite mobile at room temperature, being appreciably less viscous than sulphated oleic acid. Comparable data for a commercial sample of oleic acid are included in Table VI.

TABLE V

SULPHATION OF "PRACTICAL" ERUCIC ACID

(One hundred gram samples of "practical" erucic acid, equivalent weight 328, iodine value 67.9)

	Preparation Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Sulphation conditions												
Weight of 96% H_2SO_4, gm.	25	25	25	25	25*	25*	50	50	50	50	50	75
Time, hr., for:												
Addition of acid	1/4	1	2	2	1	2	1/4	1	1	1/2	1	1
Subsequent reaction	1	1	2	2	1	2	1	1	1	2	2	1
Analytical characteristics of sulphated oils												
Product weight, gm.	153	131	124	—	136	141	149	132	148	152	235	196
Moisture content, %	23.1	18.2	14.8	28.5	16.7	16.2	21.8	18.0	23.1	22.1	61.5	34.3
Total alkalinity, mgm.KOH/gm.	42.3	35.3	7.0	9.0	10.7	10.3	11.6	12.8	29.4	16.7	14.8	2.8
Organically combined SO_3, %	4.54	4.49	6.56	6.20	5.58	4.64	6.02	6.46	5.35	5.23	3.30	3.16
Characteristics of reconstituted fatty acids												
Melting range, °C.	75–82	66–72	65–74	68–76	65–70	55–60	51–56	52–60	40–59	50–57	42–45	40–50
Iodine value, cgm./gm.	26.6	30.7	28.2	30.8	28.8	27.2	11.4	12.5	13.9	12.4	19.0	11.9
Acid equivalent weight	378	406	424	450	442	437	655	510	492	482	674	586

* *Sulphation temperature 33°C., all other treatments at 28°C.*

TABLE VI

SULPHATION OF THE MORE UNSATURATED FATTY ACID FRACTION OF RAPESEED OIL ACIDS
(Acid equivalent weight 295, iodine value 139)

	Preparation Number					Commercial sulphated oleic acid
	13	14	15	16	17	
Sulphation conditions*						
Weight of 96% H_2SO_4, gm.	25	25	25	50	50	
Temperature, °C.	0	0	10	0	10	
Analytical characteristics of sulphated acids						
Product wt., gm.	133.6	132.2	126.8	202.5	179.4	
Moisture content, %	20.3	21.5	16.1	35.2	32.0	26.9
Total alkalinity, mgm. KOH/gm.	14.3	28.0	20.2	17.5	32.8	15.6
Organically combined SO_3, %	2.7	3.4	3.9	8.1	7.1	6.2
Characteristics of reconstituted fatty acids						
Iodine value, cgm./gm.	91.5	93.2	91.9	44.2	50.6	34.8
Equivalent weight	547	687	342	443	387	376

* *Sulphuric acid was added, during a period of one hour; thereafter the reaction mixture was held at the stated temperature for one additional hour.*

The curves of Fig. 1 indicate the effect of dilution with water on the pH of commercial sulphated oleic acid, two sulphated erucic acids, and one sulphated oil prepared from the unsaturated fraction of rapeseed oil acids. The commercial sulphated oleic acid showed slight increase in pH to a dilution of 8

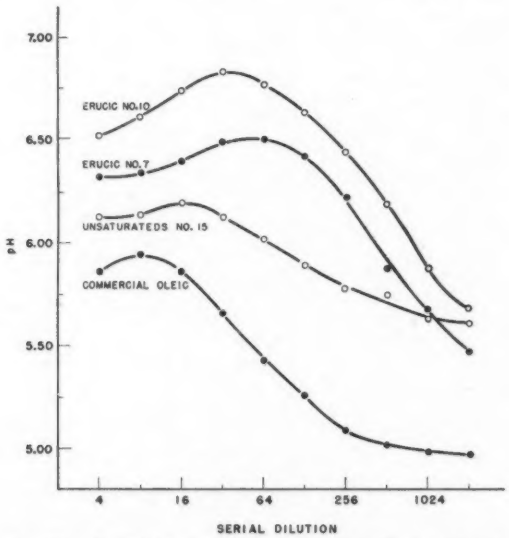

FIG. 1. *The effect of dilution with water on the pH of sulphated acids.*

(125 gm. of oil per liter of solution). The three laboratory preparations demonstrated a similar response, though the pH maxima were attained at somewhat greater dilutions.

Preparation of Hydroxy Acids and Some of Their Esters

Erucic and the more unsaturated acid fractions described in Table III were used to prepare hydroxy acids. A solution of 200 gm. "practical" erucic acid in 200 ml. *n*-heptane was cooled to 10°C., stirred mechanically while 177.5 gm. of 96% sulphuric acid was added during an hour, and held for an additional half hour at 10°C. (16). Two liters of ice water were added, and the *n*-heptane removed by steam distillation during vigorous boiling for one hour. The reaction mixture was further hydrolyzed by ethanolic potassium hydroxide, alcohol was removed, and acids liberated by dilute sulphuric acid were taken up in *n*-hexane. Various fractions of acids were obtained on cooling the solution (Table VII).

Unsaturated rapeseed oil acids (200 gm.) were dissolved in 600 ml. hexane. The solution was cooled to −5°C., 200 gm. 96% sulphuric acid was gradually added during one hour, and it was held at 0°C. for an additional half hour. Solvent removal and acid and alkaline hydrolyses were effected in the manner described above.

The iodine value, acid equivalent, saponification equivalent, and hydroxyl percentage were determined by A.O.C.S. and other methods. Melting points were observed on a Parr bar.

Ethyl esters were prepared from hydroxy acid fractions by refluxing with ethanol, using 0.75% hydrochloric acid as catalyst; washed and dried esters were distilled from a Claisen type flask at a pressure of about 0.1 mm.

Crude hydroxybehenic acid (Fraction A_1, Table VII) was polyesterified by refluxing 5 gm. in 50 ml. toluene in a system with an attached water trap (Dean-Stark). The reaction was repeated without solvent in an open reaction tube heated by the vapor of boiling ethylene glycol; a stream of carbon dioxide stirred the reaction mixture and swept away water of reaction. Ten gram samples were reacted in the same way with stoichiometrically equivalent amounts of tetradecanol–1 and octadecanol–1 obtained from the Chemical Division, Connecticut Hard Rubber Co. Wax pastes were prepared by dissolving 18 parts of the esters in 82 parts warm mineral spirits (varsol). The cooled pastes were spread on sections of hardwood benches and linoleum floors and visually observed for appearance, smear, and wear and compared in these respects with a high grade commercial wax.

Ebullioscopic molecular weights were determined by the method of Menzies and Wright (14). Cryoscopic molecular weights were determined in cyclohexane solution.

Results

Hydroxy Acids from Sulphated Practical Erucic Acid

The data of Table VII describe products (acid fractions) obtained on the hydrolysis of sulphated "practical" erucic acid and sulphated unsaturated acids from rapeseed oil. The erucic acid products (Fractions A_1–A_4) were

TABLE VII

PRODUCTS FROM HYDROLYSIS OF SULPHATED "PRACTICAL" ERUCIC ACID AND THE SULPHATED UNSATURATED ACID FRACTION FROM RAPESEED OIL ACIDS

Description	Weight of fraction, gm.*	Iodine value, cgm/gm.	Acid equivalent	Saponification equivalent	Hydroxyl, %	Melting point,** °C.
Products from hydrolysis of sulphated erucic acid dissolved in *n*-hexane						
A_1 precipitated at 8°C.	147.7	2.1	355.2	355.3	3.48	80
A_2 precipitated at −10°C.	36.5	30.0	315.8	314.8	1.14	48
A_3 precipitated at −40°C.	1.9	47.4	322.0	311.1	—	43
A_4 soluble at −40°C.	13.6	67.9	342.1	325.2	0.36	−14 to 40
Products from hydrolysis of sulphated unsaturated acids						
B_1 precipitated from *n*-hexane at 8°C.	26.7	10.7	318.9	316.1	4.33	60
B_2 soluble in *n*-hexane at 8°C.	9.9	73.6	329.4	324.4	1.85	11
Hexane insoluble material						
B_3 decanted oil (35°)	33.1	43.9	334.8	327.6	2.64	33
B_4 solid residue	136.4	37.0	329.6	321.3	3.92	49

* *Two hundred grams of each of practical erucic acid and the more unsaturated acid fraction of rapeseed oil acids were sulphated and the entire samples hydrolyzed.*

** *Fractions A_1 and A_2 melted sharply, the others over a range. The maximum value is given. A_4 was mostly oil but with a small amount of high melting material.*

soluble in warm hexane. The solid fractions A_1–A_3 were white waxy solids; A_4 was a viscous, amber oil. Analytical data suggested that Fraction A_1 was composed largely of monohydroxybehenic acid containing a little unreacted erucic acid and a small percentage of saturated acids.

Some saponifiable ester linkages are indicated by the saponification equivalents in Table VII, which, except for that of A_1, are less than the corresponding acid equivalents. Varying amounts of by-products not readily amenable to alkaline saponification are also commonly found in such reaction mixtures (3).

Hydroxy Acids from the Sulphated Unsaturated Fraction of Rapeseed Oil Acids

The unsaturated rapeseed oil acids yielded products (B_1–B_4, Table VII) that were largely insoluble in hexane. The main soluble Fraction B_1, was a white solid similar in appearance to A_1 but of lower melting point. This

fraction would appear to be a mixture of partially hydroxylated C_{18} and C_{20} acids, including some unreacted material, which was present in even greater amount in B_2, a highly viscous oil. Fractions B_3 and B_4 were separated by decantation after standing at 35°C.

Saponification equivalents of some of the fractions were redetermined using twice the usual amount of alcoholic potassium hydroxide solution and twice the usual time. Fraction B_1 (Table VII) gave a saponification equivalent of 299.7 under these circumstances; Fraction B_4 gave a value of 320.5. The lowered value for B_1 indicated further gradual saponification; little change was observed in the value for B_4.

Fractional Distillation of Ethyl Esters of Hydroxy Acids

Ethyl esters of A_1 were distilled yielding six fractions and a residue as described in Table VIII. Fractions 2 to 5 were combined and recrystallized from absolute ethanol. The white waxy solid had a m.p. of 46.6° to 47.8°C.,

TABLE VIII

VACUUM DISTILLATION OF THE ETHYL ESTERS OF HYDROLYSIS PRODUCTS OF SULPHATED "PRACTICAL" ERUCIC ACID

(Seventy-five gm. ethyl esters of Fraction A_1, Table X)

	Weight of fraction, % of charge*	Free fatty acid content calculated as mono-hydroxybehenic, %	Saponification equivalent
1	10.9	1.1	372.8
2	17.7	1.1	378.4
3	16.3	1.3	380.6
4	13.1	2.2	378.2
5	18.0	2.0	379.7
6	10.1	4.0	376.4
Residue	13.3	6.0	396.4

* *Fractions 1–4 came over with a vapor temperature of 199°C., Fractions 5 and 6 with a temperature about 225°C.*

saponification equivalent 392, hydroxyl percentage 4.14, and cryoscopic molecular weights of 383 and 384. The theoretical saponification equivalent and hydroxyl percentage for the ethyl ester of hydroxybehenic acid are respectively 384 and 4.42. While the product is largely the ethyl ester of hydroxybehenic acid, the presence of some impurities is indicated. Further unsuccessful crystallizations were made in an effort to separate the 13 and 14 hydroxy derivatives.

On distillation at reduced pressure the ethyl esters of fraction B_4, Table VII, yielded the fractions described in Table IX. The viscous polymeric residue had obviously been drastically degraded and was not investigated further. Fractions 1 to 3 contained small amounts of oily material. Fraction 4 contained a greater amount of the oily fraction while 5 was a viscous oil that did not harden at −70°C. The data indicated that substantial amounts of

TABLE IX

VACUUM DISTILLATION OF THE ETHYL ESTERS OF HYDROLYSIS PRODUCTS OF SULPHATED RAPESEED OIL UNSATURATED ACIDS

(One hundred gm. ethyl esters)

Number	Weight of fraction, % of charge	F.F.A. as monohydroxystearic, %	Refractive index, 30°C.	Saponification equivalent	Melting point,** °C.	Hydroxyl, %
1	10.8	1.2	1.4482	313.6	21	2.77
2	24.2	1.0	—	330.8	31	3.57
3	12.0	1.6	—	337.9	38	4.70
4	23.0	20.6	1.4595	331.9	27	3.19
5	10.8	35.0	1.4760	309.3	†	4.74
Recov'd residue*	15.5					

* *Viscous dark colored polymeric oil, ether extract from residue containing decomposition product.*

** *Fractions 1, 2, and 4 each melted over a range of about 4°C.; the temperature for complete fusion is given.*

† *Fraction 5 not solid at −70°C.*

monohydroxystearic acid were present in Fractions 2–4, with Fraction 3 containing the largest amount; the extent of hydroxylation of this fraction was about equal to that obtained by Roe *et al.* (16) from 97% oleic acid; a somewhat higher saponification equivalent is not surprising in view of the more complex nature of this mixture of hydroxylated acids. All five fractions were insoluble in water but soluble in petrolic ether, absolute ethanol, benzene, and carbon tetrachloride. Fractions 4 and 5 were appreciably unsaturated having iodine values, respectively, of 44.2 and 75.0. It is suggested that increased acid content (F.F.A. calculated as monohydroxystearic) in the final fractions is attributable to hydrolysis of ester on heating, water for the reaction being provided by formation of ether linkages in side reactions.

While distillation of the ethyl esters of hydroxylated rapeseed oil unsaturated acids provided substantial amounts of monohydroxystearic acid, there was considerable degradation and polymer formation.

Esters of Hydroxybehenic Acid

Crude hydroxybehenic acid (Fraction A_1, Table VII) was heated for 90 hr. in boiling toluene. The solvent-free, viscous, amber colored polyester had acid and saponification equivalents, respectively, of 1316 and 669, and the ebullioscopic molecular weight of the polymeric mixture was 1412. The reaction was repeated at 195°C. using a stream of carbon dioxide gas to sweep away the water of reaction. After 96 hr. the product was a stiff balsam with an acid equivalent of 2770 and an ebullioscopic molecular weight of 3032. Ten-gram samples of crude hydroxybehenic acid were reacted, respectively, with 7.6 gm. of octadecanol–1 and 6.02 gm. tetradecanol–1 at 195°C. After 16 hr. reaction time, hard waxy solids of light color were obtained. The octadecanol derivative had an acid number (mgm. potassium hydroxide per gm.) of 5.72

and a m.p. of 60.4°C., the tetradecanol derivative an acid number of 5.03 and a m.p. of 51. After recrystallization from petrolic ether the respective melting points were 65° and 58°C.

Pastes prepared from 18 parts of esters and 82 parts mineral spirit tended to be somewhat "watery", but spread well, giving films comparable, in hardness, sheen, and nonsmearing properties, to a high grade commercial wax coating.

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COLOR IN MARGARINE

I. EVALUATION OF COLOR USING THE LOVIBOND TINTOMETER[1]

By Audrey M. K. Brabant-Smith[2]

Abstract

The precision of color measurements, made on four brands of margarine whose color was in the vicinity of 1.6 Lovibond units of yellow and red together, was determined. A series of readings of duplicate samples of each of the margarines was made by a panel of 12 members, using a B.D.H. pattern Lovibond tintometer, and the results were subjected to statistical analysis. Observational error and individual bias both proved to be large, their net effect being to limit the accuracy of the method to ± 0.5 Lovibond units. Temperature variations also caused perceptible color changes.

Introduction

It is required by the Ontario Oleomargarine Act (2) that ". . . no oleomargarine shall have a tint or shade containing more than 1.6 degrees of yellow, or of yellow and red collectively, measured in terms of the Lovibond tintometer scale, read under conditions substantially similar to those established by the United States Bureau of Internal Revenue, or the equivalent of such measurement". A study has been made of the precision of color measurements of margarine using the Lovibond tintometer, with regard to the administration of this act. The B.D.H. pattern Lovibond tintometer (3) as currently employed in industry was used for the investigation. This instrument is an improvement on the old monocular tilting type of instrument which was specified in the United States Treasury Department Regulations covering margarine (4).

Experiments and Results

Two experiments were designed: (*a*) to determine the degree of precision that can be expected in repeated color measurements of a given sample by one observer or a group of observers, and (*b*) to study the effect of temperature on the color of margarine.

(*a*) *Precision of Color Measurements*

A panel of 12 observers was selected, on the basis of availability throughout the test and freedom from color blindness. Only one of those selected had previous experience with the use of the Lovibond tintometer, although all were experienced in some form of scientific observation; two persons had previous experience in color matching with other equipment.

Four pounds of margarine of brands sold in the Province were purchased and refrigerated at 32°F. when not actually in use. The four brands differed

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[2] *Research Fellow. Deceased August 16, 1950.*

only slightly in color, but it was possible in a suitable illumination to segregate duplicate samples by visual inspection.

The panel evaluated the same series of margarine samples repeatedly over a period of several days. Fresh duplicate samples of each of the four brands were taken daily, placed in the porcelain dishes of the tintometer, and the surface of the margarine smoothed with a spatula. Samples were presented to the observers in random order, identified only by number. The order of

TABLE I

READINGS BY PANEL OF NINE OBSERVERS

AVERAGES OF AND DIFFERENCES BETWEEN DUPLICATE PAIRS. TOTAL YELLOW AND RED UNITS

Observer	Brand No.	Trial II		Trial III		Trial IV		Trial V		Trial VI	
		Av.	Diff.	Av.	Diff.	Av.	Diff.	Av.	Diff.	Av.	Diff.
A	1	1.4	0.3	2.4	0.3	1.6	0.1	1.8	0.1	2.4	0.0
	2	1.0	0.3	1.9	0.0	1.6	0.3	1.6	0.1	1.6	0.2
	3	0.9	0.0	1.6	0.2	1.3	0.0	1.0	0.1	1.3	0.2
	4	1.2	0.1	2.4	0.0	1.8	0.1	1.7	0.2	2.2	0.1
B	1	3.2	0.1	3.0	0.1	3.5	0.0	2.8	0.1	3.1	0.2
	2	2.2	0.3	2.1	0.0	2.0	0.2	1.4	0.1	2.6	0.8
	3	2.6	0.1	2.0	0.0	2.0	0.3	1.5	0.0	2.0	0.0
	4	2.6	0.5	3.0	0.1	2.2	0.0	2.8	0.0	3.2	0.1
C	1	2.4	0.5	2.4	0.0	2.4	0.1	2.4	0.0	3.2	0.1
	2	1.6	0.0	1.5	0.0	1.4	0.3	1.4	0.1	2.0	0.6
	3	1.3	0.0	1.2	0.1	1.3	0.2	1.2	0.4	1.6	0.2
	4	1.9	0.4	1.7	0.0	2.4	0.0	2.2	0.1	2.7	0.2
D	1	2.7	0.0	2.2	0.2	2.4	0.1	2.9	0.0	2.7	0.0
	2	1.8	0.3	1.5	0.0	1.8	0.1	1.8	0.1	2.0	0.0
	3	2.0	0.4	1.2	0.0	1.5	0.2	1.7	0.0	1.6	0.1
	4	2.3	0.8	2.0	0.7	2.8	0.1	2.4	0.0	2.4	0.0
E	1	2.2	0.5	2.2	0.4	1.8	0.6	1.6	0.1	2.7	0.2
	2	2.0	0.2	2.0	0.8	1.4	0.3	1.0	0.0	2.0	0.1
	3	1.6	0.2	1.0	0.7	1.4	0.1	0.8	0.3	1.8	0.2
	4	2.0	0.7	2.4	0.4	1.6	0.1	1.1	0.2	2.9	0.6
F	1	1.9	0.4	1.5	0.0	1.6	0.1	1.6	0.2	1.6	0.1
	2	1.6	0.4	1.2	0.1	1.3	0.4	1.4	0.2	1.2	0.3
	3	1.6	0.1	1.0	0.3	1.0	0.2	1.4	0.3	1.2	0.3
	4	1.7	0.2	1.5	0.2	1.6	0.1	1.6	0.4	1.4	0.1
G	1	2.3	0.2	1.8	0.8	2.4	0.5	2.2	0.1	2.5	0.0
	2	1.6	0.0	1.4	0.2	1.5	0.2	1.4	0.1	1.8	0.4
	3	1.5	0.0	1.4	0.0	1.4	0.1	1.4	0.0	1.3	0.2
	4	2.0	0.1	1.6	0.1	2.3	0.4	2.0	0.1	2.2	0.6
H	1	2.0	0.5	2.0	0.1	2.0	0.1	2.4	0.0	2.6	0.3
	2	1.5	0.0	1.6	0.1	1.5	0.2	1.8	0.3	2.0	0.3
	3	1.5	0.2	1.5	0.0	1.6	0.3	1.6	0.0	1.8	0.3
	4	1.8	0.3	1.6	0.1	2.0	0.1	2.2	0.2	2.5	0.4
I	1	2.1	0.4	2.2	0.1	2.0	0.1	2.5	0.4	2.6	0.1
	2	1.9	0.4	1.8	0.1	2.0	0.3	2.2	0.0	2.0	0.3
	3	1.8	0.2	1.9	0.2	1.4	0.6	2.0	0.1	2.0	0.4
	4	2.1	0.0	2.2	0.5	1.5	0.0	2.4	0.5	2.2	0.4

TABLE II

ANALYSIS OF VARIANCE

	Degrees of freedom	Mean squares	Average values of mean squares	Estimated values of the components of variance	
				Components	Estimated value
Between observers	8	3.52	$\sigma^2 + 2\sigma^2stc + 8\sigma^2st + 10\sigma^2sc + 40\sigma^2s$	σ^2	0.039
Between trials	4	1.35	$\sigma^2 + 2\sigma^2stc + 8\sigma^2st + 18\sigma^2tc + 72\sigma^2t$	σ^2stc	0.013
Observers × trials interaction	32	0.49	$\sigma^2 + 2\sigma^2stc + 8\sigma^2st$	σ^2tc	0.003
Between colors	3	11.76	$\sigma^2 + 2\sigma^2stc + 18\sigma^2tc + 10\sigma^2sc + 90\sigma^2c$	σ^2sc	0.016
Observers × colors	24	0.22	$\sigma^2 + 2\sigma^2stc + 10\sigma^2sc$	σ^2st	0.013
Trials × colors	12	0.12	$\sigma^2 + 2\sigma^2stc + 18\sigma^2tc$	σ^2c	0.128
Observers × trials × colors	96	0.065	$\sigma^2 + 2\sigma^2stc$	σ^2t	0.011
Between duplicates	180	0.039	σ^2	σ^2s	0.072

NOTE: *A discussion of the estimation of variance components is given in Reference 1.*

presentation was varied from day to day, no observer participating more than once on any one day or on any one order of the samples. The first series of results obtained by each individual was discarded to allow for a period of learning the use of the instrument. The results of six sets of determinations made by each observer were recorded, each consisting of eight readings, from four pairs of duplicate samples. A statistical analysis was made of the data collected.

A preliminary study of the data obtained showed the desirability of segregating the readings of several observers for separate consideration. The results of nine of the group were considered together as being a fairly representative group that might be employed for such color measurements. Their results, together with an analysis of variance and an estimation of variance components (1) are given in Tables I and II.

Table III contains readings by observers "J", "K", "L". Because of extensive experience in color matching, observers "J" and "K" were somewhat

TABLE III

READINGS BY OBSERVERS "J", "K", AND "L"

Observer	Brand No.	Trial II		Trial III		Trial IV		Trial V		Trial VI	
		Av.	Diff.	Av.	Diff.	Av.	Diff.	Av.	Diff.	Av.	Diff.
J	1	2.4	0.3	3.1	0.0	2.2	0.0	2.2	0.0	3.0	0.0
	2	2.2	0.1	2.0	0.1	2.0	0.0	2.0	0.0	1.8	0.5
	3	2.2	0.1	2.1	0.2	2.0	0.0	1.0	0.0	1.6	0.0
	4	2.4	0.1	2.2	0.0	2.0	0.1	2.2	0.0	2.6	0.9
K	1	2.4	0.1	2.5	0.2	2.4	0.1	2.0	0.0	2.3	0.1
	2	1.8	0.1	1.6	0.0	1.6	0.0	1.8	0.0	1.9	0.0
	3	1.8	0.1	1.6	0.1	1.5	0.0	2.1	0.0	2.1	0.1
	4	2.0	0.1	2.3	0.0	2.2	0.2	2.5	0.2	2.3	0.1
L	1	1.9	1.0	2.8	0.1	1.2	0.3	2.0	1.1	2.2	0.8
	2	2.1	1.2	2.0	0.4	1.2	0.1	1.1	0.4	1.6	0.2
	3	2.1	0.4	2.0	0.3	1.1	0.2	1.0	0.3	1.5	0.2
	4	1.8	0.5	2.5	0.2	1.4	0.1	1.9	1.2	1.8	1.0

more capable in the use of the tintometer than those who lacked this experience. While their differences between duplicate readings were smaller than those in the group of nine, individual bias and variation in judgment from day to day were evident.

Observer "L" was incapable of accurate color comparison. Doubtless similar instances would be encountered in any large group of persons studied.

No attempt was made to analyze the data for yellow and red components separately in view of the inconsistency of the results when the two colors were added.

It would appear that any reading on this instrument is likely to be in error for the following reasons:

(i) No observer is capable of consistently obtaining two or more independent readings which agree exactly. This source of error is reflected in the mean square "between duplicates" (0.039). The corresponding standard deviation is $\sqrt{0.039} = 0.2$ units, closely enough. Therefore, about 30% of the color readings of an individual who has *no bias whatever* could be expected to differ from the "true" value by more than ± 0.2 units.

(ii) It appears that each individual has a "bias" which leads him to obtain readings which persistently differ from "true" value. The importance of this source of error, as exhibited by this group of nine subjects, may be judged from the magnitude of the variance associated with this component (0.072) which is large compared to the error mentioned in (i).

(iii) The change in bias of an individual from one time to another, while less important than the two sources of error mentioned above, is not inconsiderable. The value of this variance component is 0.024.

If an unspecified observer is to make color observations on margarine, the variance of error to be expected is composed of the three elements discussed above; its value is equal to the sum of these three and is therefore 0.135. Its square root, $\sqrt{0.135} = 0.37$, gives an estimate of the standard deviation of the error to be expected under these conditions. Thus one could expect about 30% of the readings to differ from the true value by more than ± 0.37 units and similarly about 10% of the readings would depart from the "true" value by more than ± 0.6 units. These percentages are calculated on the supposition that all the error components are normally distributed.

The device of increasing the number of determinations made by an observer will reduce the effects of some of these sources of error, but cannot diminish the total error to any desired level. For example, if an average be taken of five independent readings made at about the same time by a single observer, the limits ± 0.6 units quoted above would be reduced to ± 0.5 units and these limits would change little if a greater number of readings was used.

(*b*) *The Effect of Temperature on the Color of Margarine*

It was observed that the temperature within the light cabinet of the tintometer increased from 23°C. to 43°C. in a 30 min. period. To determine the effect of this temperature rise on the color of margarine, a sample from a domestic refrigerator was placed in the sample holder, the light switched on, and readings made by two observers at five-minute intervals.

The results (Table IV) show that there was a definite increase in color with increase in temperature. Even without the use of the tintometer, it could be seen that the initial "cream" color of the margarine had changed to a deep golden butter color. It is important, therefore, that color readings on margarine be taken as quickly as possible, and that the lights in the instrument be left on no longer than necessary.

TABLE IV

EFFECT ON THE COLOR INTENSITY OF MARGARINE OF A TEMPERATURE RISE* IN THE TINTOMETER CABINET

Time, min.	Lovibond units, average of duplicate readings	
	Observer 1	Observer 2
0	1.8	1.8
5	2.0	2.0
10	2.2	2.3
15	2.3	2.5
20	2.65	2.65
25	2.6	2.7
30	2.65	2.8

* *Temperature rise, 23°C. to 43°C.*

Conclusions

The evaluation of the color of margarine, using the B.D.H. pattern Lovibond tintometer, is subject to important errors from observational error, individual bias, and temperature effects. It is concluded, therefore, that this instrument is not suitable for precise evaluation of the color of margarine.

Acknowledgments

The statistical aid of Dr. D. B. DeLury, and the co-operation of the Foundation staff who participated in the trials are gratefully acknowledged.

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COLOR IN MARGARINE

II. AN INDIRECT METHOD FOR MEASUREMENT OF COLOR IN TERMS OF THE LOVIBOND COLOR SYSTEM[1]

By Audrey M. K. Brabant-Smith[2]

Abstract

A three filter photoelectric reflectometer has been adapted to measure the color of margarine. From the results so obtained the chromaticity co-ordinates can be calculated and these co-ordinates converted to Lovibond units with the aid of the Lovibond color scale conversion graph No. 3. A technique for measurement of the color of margarine is described.

Introduction

It was established in the previous paper of this series that the Lovibond Tintometer, B.D.H. pattern, could not be relied upon for consistent, reproducible results when used to determine the color of margarine (1). For the purpose of administering the Ontario Oleomargarine Act (10), it is desirable to have a method which will consistently give accurate and reproducible results for determining whether or not the color of any given sample of margarine is within the legal limits. It is desirable also that the results obtained be convertible into Lovibond units.

Selection of a Colorimeter

A Hunter multipurpose reflectometer (4, 5) was selected for measurement of margarine color. It is a three filter photoelectric colorimeter and the measurements can be readily expressed in terms of the I.C.I. trichromatic system (2, 6, 8, 9). A study of the use of this instrument for tristimulus colorimetry has been made by the staff of the Institute of Paper Chemistry (7).

Photoelectric tristimulus colorimeters are not completely satisfactory as they do not exactly duplicate the I.C.I. standard observer. However, for the comparison of near-white surfaces the degree of duplication is sufficient (5, page 28). As margarine colors closely resemble "whites", an instrument of this type should be satisfactory for their measurement.

Conversion of Chromaticity Co-ordinates to Lovibond Units

The Tintometer Ltd. have prepared graphs for conversion of the Lovibond color scale to chromaticity co-ordinates. Graph No. 3 has been computed with respect to Standard Illuminant C as is used in the Hunter reflectometer

[1] *Manuscript received in original form August 14, 1950, and as revised, January 24, 1951. Contribution from the Department of Biochemistry, Ontario Research Foundation, Toronto, Ont.*

[2] *Research Fellow. Deceased August 16, 1950.*

TABLE I

CALIBRATION OF LOVIBOND GLASSES AGAINST VALUES FROM CONVERSION GRAPH

Chromaticity co-ordinates		Values of Lovibond glasses										
		8.0	6.0	4.0	3.0	2.0	1.6	1.0	0.6	0.5	0.3	0.1
		Yellow glasses										
Calculated from reflectometer readings	x	0.389	0.377	0.361	0.353	0.340	0.336	0.327	0.322	0.318	0.314	0.310
	y	0.437	0.424	0.397	0.384	0.375	0.359	0.345	0.332	0.331	0.324	0.321
From Lovibond conversion graph	x	0.390	0.379	0.362	0.352	0.341	0.335	0.326	0.320	0.318	0.316	0.312
	y	0.459	0.441	0.413	0.398	0.374	0.365	0.349	0.338	0.335	0.329	0.322
		Red glasses										
Calculated from reflectometer readings	x	0.394	0.371	0.350	0.340	0.331	0.325	0.321	0.319	0.317	0.315	0.311
	y	0.277	0.275	0.286	0.291	0.296	0.301	0.306	0.309	0.311	0.312	0.316
From Lovibond conversion graph	x	0.383	0.364	0.344	0.335	0.327	0.324	0.319	0.315	0.314	0.313	0.311
	y	0.277	0.282	0.290	0.295	0.302	0.305	0.309	0.313	0.314	0.315	0.317

(11). Haupt and Douglas have also published graphs giving the chromaticity co-ordinates of certain Lovibond glasses (3). Their values essentially agree with those of The Tintometer Ltd.

A set of 2 in. × 3/4 in. Lovibond glasses was calibrated directly with the Hunter reflectometer in terms of chromaticity co-ordinates in order to ascertain how close these values were to the true values read from the conversion graph. Owing to the small size of the glasses it was necessary to attach them by means of adhesive tape over the small aperture in front of the fixed photocell. The values obtained, given in Table I, were close to those read from the conversion graph only in the near-white range. As the colors increased in saturation the values for *y* were progressively lower than those read from the conversion graph.

The relationship between Lovibond values in the near-white range and chromaticity co-ordinates is shown in Fig. 1. The apices of the triangle A B C represent I.C.I. white, 1.6 yellow units, and 0.9 yellow plus 0.7 red units respectively, and the color limits as stated in the Oleomargarine Act lie within this area. At any point along the line B C the sum of yellow and red units

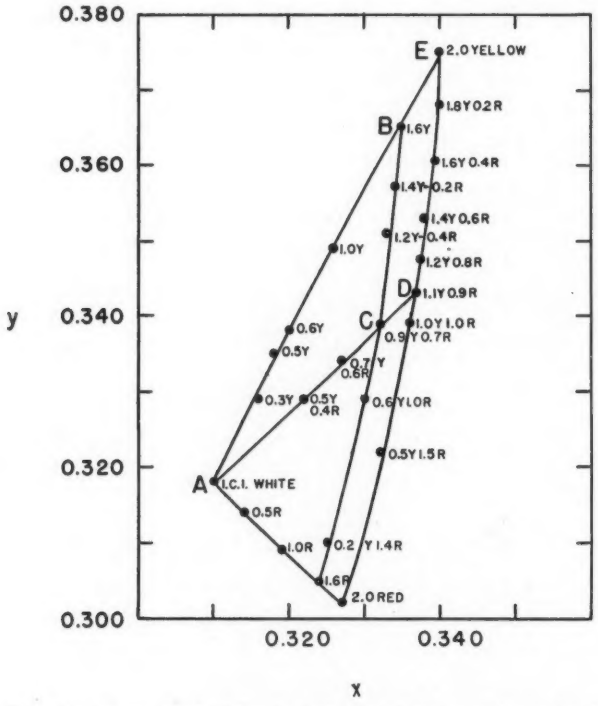

FIG. 1. *Relationship between Lovibond units and chromaticity co-ordinates for Standard Illuminant C.*

is 1.6, and within the rectangle B C D E the color is in excess of 1.6 units but not greater than 2.0 units. Below the line A D, red exceeds yellow. Along the line A E, corresponding to pure yellow, an increase of 0.0015 x and 0.0030 y corresponds to an increase of 0.1 Lovibond units, but along the line A D an increase of 0.0013 x and 0.0013 y is approximately equivalent to 0.1 Lovibond units.

Technique of Color Measurements on Margarine

In initial experiments small samples of margarine were put in Petri dishes and covered with a glass in close contact with the margarine. This procedure was unsatisfactory, however, as light penetrated through such a sample, and it was difficult to fill the Petri dishes without entrapping air bubbles which affected the readings. It was found to be more satisfactory to remove the wrapper from one side of a pound or half-pound print and cover the exposed side with a square of glass in such a way that no air bubbles were entrapped. This provided a smooth uniform surface, which is necessary for consistent color measurements, and at the same time prevented the instrument from being greased with margarine when the sample was placed in the reflectometer sample holder. The glass squares were cut from the same sheet and polished at the edges. The amount of color in the glass was small, and was compensated for by placing one of the covers over the standard used for adjusting the instrument.

In practice, the instrument was kept in a conditioned room at 70°F. and 20% relative humidity. It was adjusted with one of the glass covers over the ceramic secondary standard to read the standard value of the tile in green light. The filters were then changed and readings of the standard plus glass noted in amber and in blue light. The margarine sample was now put in position and readings were noted in green, amber, and blue light. The standard values of the tile in amber and in blue light were divided by the scale reading of the standard plus glass in amber and in blue light, and these factors were used to correct the corresponding readings of the margarine sample for the color of the glass cover.

The tristimulus values X, Y, and Z were then calculated by means of the following equations (5, page 12):

$$X = 0.80\,A + 0.18\,B \quad (1)$$

$$Y = 1.00\,G \quad (2)$$

$$Z = 1.18\,\mathrm{B} \quad (3)$$

where A, B, and G were the corrected readings for the amber, blue, and green filters respectively. The relationship between the chromaticity co-ordinates and the tristimulus values is as follows:

$$x = \frac{X}{X + Y + Z} \quad (4)$$

$$y = \frac{Y}{X + Y + Z} \quad (5)$$

$$z = \frac{Z}{X + Y + Z} \quad (6)$$

The chromaticity co-ordinates were calculated by substituting the value of X, Y, and Z obtained from Equations 1, 2, and 3 in Equations 4, 5, and 6. As the sum of the chromaticity co-ordinates is unity, specification of any two co-ordinates fixes the third.

To test the reproducibility of color measurements by this method readings were made from day to day on a pound of margarine with one side covered with a glass plate in the manner already described. The margarine was stored at 22°F., removed from storage each day, and immediately assessed for color, then replaced in storage. The calculated chromaticity co-ordinates are given in Table II. The results agree to within 0.001 in each of the three co-ordinates and indicate that the instrument is consistent to this limit.

Since results obtained with the Hunter reflectometer are reproducible to 0.001 in each of the three co-ordinates, and since this is the maximum accuracy with which the conversion graph may be read, this method is accurate and reproducible to ±0.1 Lovibond units. It was established previously that the accuracy of readings with the Lovibond tintometer was limited to 0.5 units (1).

To illustrate the application of the method, the values obtained for a number of samples of margarine and for one sample each of lard and shortening are given in Table III. The approximate Lovibond values corresponding to the chromaticity co-ordinates and read from Lovibond Conversion Graph No. 3 are given in the last column of this table. If it were only required to know whether or not the margarine color was within the legal limits, it would be sufficient to determine whether or not the x and y values were within the triangle A B C of Fig. 1.

The Effect of Temperature of Color Measurements

One pound of each of three brands of margarine was covered on one side with a glass and conditioned for 24 hr. periods at 32°F., then at 60°F., 70°F., and finally again at 32°F. Color measurements were made after each 24 hr. period, and the results are given in Table IV. It will be observed that there was an increase in the x and y values when readings were made after conditioning at the higher temperatures. However, after again conditioning the margarine at 32°F., the color values were essentially the same as those obtained after the first conditioning at that temperature. The maximum change in color from 32°F., to 70°F., was about 0.003 for x and y, or an increase of 0.2–0.3 Lovibond units.

TABLE II

DAILY COLOR READINGS ON ONE SAMPLE OF MARGARINE

Days	Chromaticity co-ordinates	
	x	*y*
1	0.3407	0.3560
3	0.3413	0.3559
4	0.3404	0.3565
5	0.3410	0.3565
6	0.3411	0.3557
7	0.3412	0.3554
8	0.3411	0.3557

NOTE: *The sample was refrigerated between readings.*

TABLE III

MARGARINE COLOR MEASURED IN TERMS OF I.C.I. CHROMATICITY CO-ORDINATES, AND EXPRESSED AS APPROXIMATE EQUIVALENT IN LOVIBOND UNITS

Sample	Chromaticity co-ordinate		Approximate total Lovibond units from Fig. 1 and conversion graph
	x	*y*	
No. 1	0.344	0.354	2.4
No. 2	0.339	0.348	1.9
No. 3	0.329	0.338	1.4
No. 4	0.327	0.337	1.2
No. 5	0.339	0.350	1.8
No. 6	0.340	0.348	2.2
No. 7	0.328	0.338	1.2
Lard	0.318	0.322	1.2 red exceeds yellow
Shortening	0.331	0.340	1.4

TABLE IV

EFFECT OF TEMPERATURE ON COLOR OF MARGARINE

Samples	Conditioned 24 hr. at:			
	32°F.	60°F.	70°F.	32°F.
No. 1				
x	.340	.342	.343	.340
y	.345	.347	.348	.344
No. 2				
x	.335	.337	.339	.335
y	.344	.346	.347	.345
No. 3				
x	.345	.348	.349	.345
y	.352	.354	.355	.352

A temperature of 60°F. was found convenient. This temperature was adopted for all color measurements, and all samples were conditioned at this temperature prior to color measurements being made.

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COLOR IN MARGARINE

III. EFFECT OF STORAGE CONDITIONS[1]

By Audrey M. K. Brabant-Smith[2]

Abstract

Changes in the color of margarine brought about by storage for various lengths of time at different temperatures were studied. The relationship between type of wrapper and brand of margarine was also investigated. No important color changes were observed except where parchment wrapping was employed. In this case a general increase in color occurred owing to surface dehydration of the margarine. Exposure of wrapped margarine to ultraviolet irradiation and to atmospheres of oxygen or nitrogen had no perceptible effect.

Introduction

A study of the literature on the subject of alteration in the color of butter, margarine, or shortening during storage furnished little specific information; some authors claimed that color was affected by storage, but little or no evidence was submitted to substantiate these statements. In view of the lack of information, a quantitative study has been made of the possible color changes in margarine resulting from the conditions of storage since such questions must inevitably arise in the administration of the Ontario Oleomargarine Act (5).

Materials and Methods

Samples of margarine manufactured from a variety of blended oils by four Ontario manufacturers were selected for the test. The brands were coded A, B, C, and D and the wraps in a different order, Nos. 1, 2, 3, and 4. Wrap No. 3 was parchment and wraps Nos. 1, 2, and 4 were impermeable.

The assessment of color was made with the use of a Hunter reflectometer by the method described in the second paper of this series (1). The error of measurements was small, the standard error of both x and y co-ordinates being about 0.0013, corresponding approximately to 0.1 Lovibond units.

Experiments and Results

Effect of Storage Conditions on Color

For this study 148 1-lb. packages of each of the four brands of margarine were obtained. The four lots were subdivided into four groups of 37 packages each. One group of each brand was left in its own wrappers. The wrappers were removed from the remaining groups and these were then rewrapped in the laboratory so that each brand could be stored in the other types of wrapper as well as in its own.

[1] *Manuscript received in original form August 14, 1950, and as revised January 24, 1951. Contribution from the Department of Biochemistry, Ontario Research Foundation, Toronto, Ont.*

[2] *Research Fellow. Deceased, August 16, 1950.*

The margarine was stored at 32°F., 40°F., 60°F., and 70°F. Color measurements were made at intervals up to 70 days for all samples, except those stored at 70°F. The latter deteriorated so badly in flavor and odor that they were discarded 20 days after the commencement of the test. The results are summarized statistically in Tables I to VIII.

TABLE I

ANALYSIS OF VARIANCE OF x CO-ORDINATE

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F ratio
Between brands	3	728803.42	242934.47	1708.28
Between wraps	3	15785.28	5261.76	37.00
Brands × wraps	9	10058.48	1117.61	7.86
Times	4	669.08	167.27	1.18
Brands × times	12	7808.75	650.73	4.58
Wraps × times	12	2813.06	234.42	1.65
Temperatures	2	2155.30	1077.65	7.58
Brands × temperatures	6	1856.43	309.41	2.18
Wraps × temperatures	6	1157.87	192.98	1.36
Times × temperatures	8	1731.25	216.41	1.52
Error	174	24545	142.21	
Total	239	797584		

TABLE II

SELECTED CONTRASTS FROM TABLE I

Selected contrasts		Degrees of freedom	Sums of squares	
Between brands	(a) C and D contrasted with A and B	1	305877.60	728803.92
	(b) C and A contrasted with D and B	1	89375.00	
	(c) C and B contrasted with D and A	1	338550.82	
Between wraps	(α) 1, 2, and 4 contrasted with 3	1	14346.94	15785.28
	(β) 1 and 2 contrasted with 4	1	1396.34	
	(γ) 1 contrasted with 2	1	42.01	
	($a\alpha$)	1	200.56	10058.50
	($b\alpha$)	1	924.80	
	($c\alpha$)	1	7156.81	
	($a\beta$)	1	73.80	
	($b\beta$)	1	24.03	
	($c\beta$)	1	95.07	
	($a\gamma$)	1	31.01	
	($b\gamma$)	1	1222.41	
	($c\gamma$)	1	330.01	

TABLE III

ANALYSIS OF VARIANCE OF *y* CO-ORDINATE

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F ratio
Between brands	3	939488.23	313162.74	1531.43
Between wraps	3	28589.76	9529.92	46.60
Brands × wraps	9	17309.07	1923.23	9.41
Times	4	2625.14	656.29	3.21
Brands × times	12	9690.22	807.52	3.95
Wraps × times	12	5180.53	431.71	2.11
Temperatures	2	2408.05	1209.03	5.89
Brands × temperatures	6	1705.15	284.19	1.39
Wraps × temperatures	6	2206.92	367.82	1.80
Times × temperatures	8	8196.24	1024.53	5.01
Error	174	35582	204.49	
Total	239	1052981		

TABLE IV

SELECTED CONTRASTS FROM TABLE III

Selected contrasts	Degrees of freedom	Sums of squares	
Between brands			
(a) C and D contrasted with A and B	1	356818.82	
(b) C and A " " D and B	1	93062.82	939488.31
(c) C and B " " D and A	1	489606.67	
Between wraps			
(α) 1, 2, and 4 " " 3	1	27429.36	
(β) 1 and 2 " " 4	1	840.28	28589.77
(γ) 1 " " 2	1	320.13	
(aα)	1	708.05	
(bα)	1	1253.47	
(cα)	1	14400.56	
(aβ)	1	14.40	
(bβ)	1	16.04	17308.99
(cβ)	1	31.21	
(aγ)	1	100.83	
(bγ)	1	288.30	
(cγ)	1	496.13	

Discussion and Interpretation of the Tables

Tables I and III show analyses of variance (3) of the color changes in the *x* and *y* co-ordinates. The large "between wraps" mean squares show that color changes took place during the experiment and the presence of substantial "brands × wraps" interactions indicates that differences between wraps were not uniform over the four brands.

Tables II and IV represent an attempt to identify the sources of variation whose effects are displayed in the large "between wraps" and "brands ×

wraps" mean squares. The large components (α) indicate that most of the differences among wraps lie in the difference between Wrap 3 and Wraps 1, 2, and 4 and the large components ($c\alpha$) show that most of the "brands × wraps" interaction comes from the failure of the difference between Wrap 3 and Wraps 1, 2, and 4 to take the same value on Brands C and B as it does on Brands A and D. Component (β) is, in both tables, significantly large, indicating that Wrap 4 is detectably different, in permitting color change, from Wraps 1 and 2. Component (γ) is, in both tables, far from significant and we may, within the limits of this experiment, regard Wraps 1 and 2 as identical in so far as they permit color changes of margarine. These effects may be seen in Table V.

Returning to Tables I and III, the "times" mean squares give no conviction of a persistent time effect. The "times" mean square is definitely not significant for the x co-ordinate and is just over the 5% point for the y co-ordinate. The only term, other than those already discussed, that seems to merit attention is "temperatures" which is strongly significant for both co-ordinates. Reference to Tables VI and VII reveals that temperature differences were almost entirely due to a fall in the color readings of samples stored at 40°F. No explanation of this curious effect can be offered with confidence. In any case, the effect is small and apparently of no particular importance.

Since color changes depend on particular combinations of brands and wraps, it seems appropriate to study color variation when each brand is stored in its own wrap. Table VIII provides an analysis of variance for this portion of the data. None of the mean squares in this table indicates appreciable effects except temperature, which shows the same effects as were mentioned above.

Effect of Oxidation and of Dehydration on Color

Samples of each brand of margarine in the various wrappings were placed in each of four desiccators in atmospheres of oxygen, nitrogen, air at 100% and at 0% relative humidity respectively. The storage temperature was 70°F. Color measurements were made after 10 days and 20 days. No significant color change due to oxidation was noted in any of the samples. However, dehydration caused a marked surface darkening in margarine wrapped in parchment (wrap No. 3). The other types of wrappings protected the margarine against dehydration and consequently prevented a color change.

On repeating the test at 0% relative humidity, an intensification of the color was visible through the parchment wrapping after four days' exposure. The co-ordinates x and y increased in 10 days by 0.0078 and 0.0110 respectively, which is approximately equal to 0.6 Lovibond units of yellow and red. Control samples at 100% relative humidity showed no color change.

It is apparent from these results that the parchment wrapping is less suited than other types tested for the maintenance of initial color and moisture content of margarine. Similar effects have been observed by White (4) in butter stored in parchment wrappings.

TABLE V

AVERAGES, BRANDS, AND WRAPS

Brands	Wraps								Overall averages	
	1		2		3		4			
	x	*y*	*x*	*y*	*x*	*y*	*x*	*y*	*x*	*y*
A	.3393	.3508	.3398	.3512	.3405	.3519	.3402	.3512	.3400	.3513
B	.3429	.3551	.3428	.3557	.3455	.3589	.3437	.3559	.3437	.3564
C	.3294	.3396	.3284	.3390	.3293	.3400	.3291	.3398	.3291	.3396
D	.3387	.3507	.3397	.3516	.3431	.3565	.3399	.3517	.3403	.3526
Overall av'gs	.3376	.3490	.3377	.3494	.3396	.3518	.3382	.3497	.3383	.3500

TABLE VI

AVERAGES, WRAPS, AND TEMPERATURES

Wraps	Temperatures, °F.						Averages	
	32°		40°		60°			
	x	*y*	*x*	*y*	*x*	*y*	*x*	*y*
1	.3377	.3490	.3373	.3488	.3377	.3493	.3376	.3490
2	.3375	.3492	.3375	.3493	.3380	.3496	.3377	.3494
3	.3402	.3525	.3388	.3508	.3399	.3522	.3396	.3518
4	.3384	.3501	.3378	.3493	.3385	.3496	.3382	.3498
Averages	.3384	.3502	.3378	.3495	.3385	.3502	.3383	.3500

Note the rather persistent, small drop in both color co-ordinates from 32° F. to 40° F. then a rise at 60° F. to virtual equivalence with the 32° F. reading. This is most marked with No. 3 and No. 4 wraps.

TABLE VII

AVERAGES, WRAPS, AND TEMPERATURES

Brands	Temperatures, °F.						Averages	
	32°		40°		60°			
	x	*y*	*x*	*y*	*x*	*y*	*x*	*y*
A	.3395	.3510	.3400	.3512	.3404	.3517	.3400	.3513
B	.3440	.3567	.3432	.3561	.3440	.3564	.3437	.3564
C	.3294	.3399	.3286	.3391	.3292	.3399	.3291	.3396
D	.3409	.3533	.3396	.3518	.3404	.3528	.3403	.3526
Averages	.3384	.3502	.3378	.3495	.3385	.3502	.3383	.3500

As in Table VI slight drops at 40° F. are noticeable with brands B and D.

TABLE VIII

ANALYSIS OF VARIANCE OF COLOR CHANGES OF EACH BRAND IN ITS OWN WRAP

Source of variation	Degrees of freedom	*x* Co-ordinate		*y* Co-ordinate	
		Sums of squares	Mean squares	Sums of squares	Mean squares
Brands	3	195099.52	65033.17	236281.80	78760.60
Times	4	240.55	60.14	848.45	212.11
Brands × times	12	1583.31	131.94	2136.61	178.05
Temperatures	2	1223.20	611.60	1288.24	644.12
Brands × temperatures	6	327.20	54.53	650.16	108.36
Times × temperatures	8	499.47	62.93	2219.76	277.47
Error	24	2437.47	101.56	4025.18	167.72
Total		201410.72		247450.20	

Effect of Ultraviolet Light on Color

Unwrapped portions of each of the four selected brands were placed side by side under Corning filter No. 9863, transmitting in the range 220–420 mμ (3) and illuminated with a mercury vapor lamp placed 8 in. above the filter for 30 min. One-half of each sample was covered by a strip of heavy aluminum foil so that any color change would be readily apparent in the unprotected surfaces of the margarine.

Increase in total color was noted in each case in the exposed parts of the margarine after one hour's irradiation. When the experiment was repeated using Corning filter No. 5874 with the narrower ultraviolet range of 300–410mμ, increase in color was again noted after one hour's irradiation but this was considerably less marked than when the broad band filter was used. Even by doubling the exposure time the color was less than in the previous trial.

Margarine in each of the three types of wrapping was next irradiated through Corning filter No. 5874 for eight hours. As in the experiment with unwrapped margarine, one-half of the sample was protected against irradiation by a heavy grade of aluminum foil. The temperature of the margarine during irradiation was observed by means of a thermometer inserted into the center of the sample under the exposed surface. Air circulation was adjusted so that the temperature did not rise above 75°F. None of the margarine samples showed any color change where protected by the wrapper during exposure. The plastic wrapping was itself considerably darkened, but it continued to protect the margarine effectively.

Conclusions

None of the four brands tested showed important color changes when stored in their own wraps up to 70 days at temperatures between 32°F. and 60°F. At 70°F. all samples deteriorated seriously in flavor and odor before any significant change in color was noted. Color increase during storage

occurred on some brands when parchment was used for wrapping, owing to surface dehydration of the margarine. Ultraviolet light also caused a color change in margarine but such an effect is unlikely under normal conditions of storage where the product is marketed in a cardboard container with an inner wrapper.

Addendum

A referee has drawn attention to the fact that the experimental procedure was not wholly uniform, inasmuch as samples tested in their own wrappers were left in the wrappers originally put on in the factory, while those tested in other wrappers were unwrapped and rewrapped by hand.

It seems not at all unlikely that unwrapping and rewrapping a sample would have some effect on the ultimate color reading, but it is to be remarked that this effect, if present, would show up only in brands × wraps interaction. All other comparisons would be unaffected by it.

A study of the data indicates that neither co-ordinate could have been affected by more than about 0.001 units by unwrapping and rewrapping.

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